Biosynthesis of Silver Nanoparticles (AgNPs) using plant material as nano-drug against pathogenic bacteria

A SYNOPSIS OF RESEARCH WORK PROPOSED TO BE CARRIED OUT IN PURSUANCE OF THE REQUIREMENT FOR THE AWARD OF DEGREE

OF

DOCTOR OF PHILOSOPHY

IN

BOTANY

SUBMITTED

BY

ANSHU SINGH

Prof. J.N. Srivastava
Supervisor
Department of Botany

Prof. D.S. Rao
Head
Department of Botany

Prof. Ravindra Kumar
Dean
Faculty of Science

Department of Botany, Faculty of Science,
Dayalbagh Educational Institute (Deemed University)
Dayalbagh, Agra-282005

(September, 2015)

INTRODUCTION

In the recent time, Nanoscience and Nanotechnology are provoking substantial interest due to wide range of applications in the field of catalysis, medicine, bio-sciences, drug delivery, dwindling of pollution etc. Metal and metal oxide nanoparticles are intensively developed because of their importance both for fundamental science and advanced technology. Silver nanoparticles are of great importance in the current research areas among different metal based nanoparticles. Silver nanoparticles can be synthesized by various methods namely, thermal decomposition of silver compounds (Plante *et al*. 2010), radiation directed (Li *et al*. 2011), microwave directed (Nadagouda *et al*. 2011) and sonochemical methods.

Nanoparticles synthesized using biological systems are referred to as biogenic nanoparticles. The biological methods of nanoparticles synthesis using biological resources including bacteria, yeast, fungi (Ahmad *et al*. 2002, Ahmad *et al*.2003, Shahverdi *et al*. 2007, Jha *et al*. 2009, Bharde *et al.* 2008) and plants (Lee *et al*. 2010, Torresdey *et al*. 2003) have already been reported as clean, innocuous and economically satisfactory ambiant routes. The use of plant extracts for synthesis of nanoparticles is potentially advantageous over microorganisms due to the ease of scale up, less biohazard and elaborate process of maintaining cell culture. The biogenic synthesis of nanoparticles opens a new possibility of conveniently synthesizing pure metallic nanoparticles using natural products (Manisha *et al*. 2013). A study on previous reports of nanoparticle synthesis supports the simple, rapid and economical green route synthesis of AgNPs using leaf extract, fruit extract, flower extract, seed extract and micro-organisms with eco-friendly manner and their capability of rendering the antimicrobial efficacy (Karunakar *et al*. 2012, Koyyati *et al*. 2014, Koyyati *et al*. 2013, Nalvothula *et al*. 2014).

In this regard, we will make an attempt to synthesize silver nanoparticles using seeds powder of *Carica papaya* (papaya), *Cucurbita pepo* (pumpkin) and *Momordica Charantia* (bittergourd). As the pathogenic microbes are gaining strong multi-drug resistance capability, consequently the use of antibiotics has become restricted. For overcoming this problem the use of natural plant products is preferred over the synthetic antibiotics. Thus for this purpose the antimicrobial activity of the prepared plants seed powder extract will be measured against selected pathogens including bacteria- *Pseudomonas*

aeruginosa, *Bacillus subtilis*, *Bacillus fusiformis, Pseudomonas flourescens, Proteus vulgaris* and *Enterobacter aerogenes.* The seed powder extract synthesizing most potent silver nanoparticles and showing maximum bactericidal effect among the three plants seed powder extract will be selected for further analysis.

HISTORICAL BACKGROUND OF NANOTECHNOLOGY-

The concepts of nanotechnology were first discussed by renowned physicist Richard Feynman in his talk "There's Plenty of Room at the Bottom" in 1959, in which he described the possibility of synthesis via direct manipulation of atoms. Norio Taniguchi (1974) coined the term "nanotechnology". Thereafter the term "nanotechnology" was used by K. Eric Drexler in his book "Engines of Creation: The Coming Era of Nanotechnology" published in 1986, in which he proposed the idea of a nanoscale "assembler" which would be able to build a copy of itself and of other items of arbitrary complexity with atomic control. Challenges were evoked for understanding the applications of molecular nanotechnology, which became a topic of public debate between Drexler and Smalley in 2001 and 2003 (Drexler and Smalley, 2003).

BIOSYNTHESIS OF NANOPARTICLES

Nanoparticles biosynthesis is a type of bottom up approach where the main reaction occurring is reduction/oxidation. Reduction of metal compounds into their respective nanoparticles is mediated by the microbial enzymes or the plant phytochemicals with anti-oxidant or reducing properties (Raveendran *et al.* 2003). The best method adopted for nanoparticles synthesis is the use of bio-organisms which shows compatibility with the green chemistry principles: Since bio-organism possess the following properties: (i) they are environment friendly (ii) they act as reducing agent (iii) they are the capping agent in the reaction (Li *et al*. 2007). There are two ways by which metal nanoparticles can be prepared; the first one comprises physical approach that includes several methods such as laser ablation and evaporation or condensation while the second one comprises chemical approach in which the formation of small metal aggregates is facilitated by the reduction of metal ions in solution.

USE OF PLANTS TO SYNTHESIZE NANOPARTICLES

Plants are being used as traditional medicines since ancient times as they are known to possess various therapeutic compounds. Plants have huge diversity thus can be ashored for wide range of applications in the field of agricultural, pharmaceutical, industrial etc. A wide range of biomolecules such as alkaloids, terpenoids, phenols, flavanoids, tannins, quinones etc. have been evaluated to mediate synthesis of nanoparticles. These metabolites may aid in reduction. The plants are found to be advantageous over other resources because they are easily accessible, extremely easy and safe to handle. A wide range of plants have been currently investigated for their vital role in the synthesis of nanoparticles.

Characteristics of selected plant

1. *Carica papaya* L.-

Classification-

Kingdom	**Plantae**
Unranked	**Angiosperms**
Unranked	**Eudicots**
Unranked	**Rosids**
Order	**Brassicales**
Family	**Caricacae**
Genus	***Carica***

The papaya is a large, usually unbranched, tree-like plant, with a single stem growing from 5 to 10 m tall, with leaves having spiral arrangement that confine to the top of the trunk. When the fruit feels to be soft and its skin has attained amber to orange colour, it is said to be ripen. Papaya is native to northern, southern and Central America and other parts of the world also.

Papayas can be used as a food, a cooking aid and in traditional medicine (Morton, 1987). Its stem and bark may exclusively be used in rope production. Its seeds contain high levels of proteolytic enzymes like papain which can help our body to get rid of parasites. Papain breaks down undigested protein waste in our food, it may also break down parasites and their eggs. Enzyme rich green papaya capsules are also available that are the alternative to fresh fruit. A unique anthelmintic alkaloid called carpaine is present in the seeds of papaya that are found to be very effective against parasitic worms and amoebes. Also the papaya seeds can be used with milk to avoid typhoid disease and to cure from hemorrhoids-kind diseases. A special compound is also reported in papaya seeds to stop formation of tumor. Seeds are reported to have various phytochemicals and Vitamin A, C & E that provides antioxidant protection.

2. *Cucurbita pepo* L. –

Classification:-

Kingdom	**Plantae**
Clade	**Angiosperms**
Clade	**Eudicots**
Clade	**Rosids**
Order	**Cucurbitales**
Family	**Cucurbitaceace**
Genus	***Cucurbita***

Cucurbita pepo belongs to the genus *Cucurbita*. Varieties of winter squash and pumpkin are yielded by it; most of the varieties belong to *Cucurbita pepo* subsp. *pepo*, called summer squash. The plants have yellow flowers and are typically 1.0-2.5 feet high and 2-3 feet wide.

Pumpkin seeds contain linoleic acid as the main component alongwith fatty acids. The high tryptophan content of the seeds may make the oil useful in cases of insomnia. Seeds are also found helpful for people with acute schistosomisis, a severe parasitic disease that is transmitted through snails. They improve the function of the bladder and urethra and act as potent diuretic. These are a very good source of the minerals like phosphorus, magnesium, manganese, zinc, iron and copper. Additionally, they are a good source of vitamin K and protein. They can also be used as an anthelmintic and taeniacide.

3. *Momordica charantia* L.-

Classification-

Kingdom	**Plantae**
Clade	**Angiosperms**
Clade	**Eudicots**
Clade	**Rosids**
Order	**Cucurbitales**
Family	**Cucurbitaceace**
Genus	***Cucurbita***

Momordica charantia is also known as bitter melon, bitter gourd or bitter squash. It is widely grown in Asia, Africa and Caribbeana and is tropical and subtropical vine of the family Cucurbitaceae. Many of its varieties differ considerably in the shape and bitterness of the fruit. The fruit has a relevant shape and distinct warty exterior. On observing its cross-section it is hollow, with a central seed cavity filled with large, flat seeds and pith surrounded by a thin layer of flesh. *Momordica charantia* (bitter melon) seed oil contains approximately 60% Eleostearic acid (alpha-ESA) which is a conjugated linolenic acid. Eleostearic acid (alpha-ESA) is found to block breast cancer cell proliferation and induce apoptosis (Grossmann *et al*. 2009).

TARGET PATHOGENS

1) *Bacillus fusiformis:* *B. fusiformis* is a Gram positive, long rod-shaped bacteria measuring 5 to 10 by 0.6 to 0.8 µ, it s slightly swollen in the middle and pointed at the ends. It takes stain with the ordinary dyes readily. It has been reported to occur in cases of hospital gangrene. It is found more commonly at present in the form of Vincent's angina which is an inflammatory condition of the throat and it is known to be the casual agent of Vincent gingivitis. Symptoms of the disease include swollen and bleeding gums, small, painful ulcers covering the gums and tooth margins and characteristic fetid breath. The ulcers may spread to the throat and tonsils. It is found exclusively in the toxin of puffer fish called tetrodotoxin, which is a highly fatal neurotoxin that destroys the central nervous system of humans causing paralysis.

2) *Bacillus subtilis:* *B. subtilis* is a Gram-positive, catalase-positive, rod-shaped bacterium. It is also known as the hay bacillus or grass bacillus. It is found in soil and the gastrointestinal tract of ruminants and humans. *B. subtilis* can form a tough, protective endospores that allow it to cope up with the extreme environmental conditions. Historically *B. subtilis* has been classified as an obligate aerobe, though it is evidenced as facultative aerobe. *B. subtilis* is considered the best studied Gram-positive bacterium and a model organism to study bacterial chromosome replication and cell differentiation (Ferdinand, 1872). *B. subtilis* contaminate food and causes food poisoning.

3) *Enterobacter aerogenes:* *E. aerogenes* is a pathogenic bacterium; it is a Gram negative, oxidase negative, citrate positive, hospital acquired infection. It causes opportunistic infections including most types of infections. These bacteria swiftly gain resistance against standard antibiotics during treatment which requires the change in antibiotic to avoid exacerbating of the sepsis (Sanders *et al*. 1997)

It causes various infections including urinary tract infections, bacteremia, skin and soft tissue infections, lower respiratory tract infections, endocarditis, intra-abdominal infections, septic arthritis, osteomyelitis, and ophthalmic infections.

4) *Proteus vulgaris:* *P. vulgaris* is a Gram-negative, rod-shaped, catalase-positive, nitrate-reducing, indole positive and hydrogen sulfide-producing bacterium. It inhabits the intestinal tracts of humans and

animals. It can be found in soil, water and fecal matter. It is an opportunistic pathogen of humans and is grouped with the Enterobacteriaceae . It is causal agent of urinary tract infections and wound infections.

5) *Pseudomonas fluorescens*: *P. fluorescens* is a common rod-shaped Gram-negative, bacterium (Palleroni *et al*. 1984). *P. fluorescens* produce heat-stable lipases and proteases and other similar Pseudomonads (Frank *et al.* 1997). The enzymes produced by this bacterium causes bitterness of milk and spoil it, this is facilitated by breakdown of casein and ropiness due to production of slime and coagulation of proteins (Jay, 2000 and Ray, 1996). *P. fluorescens* has been known to infect blood transfusions due to its hemolytic activity (Gibb *et al.* 1995).

6) *Pseudomonas aeruginosa:* *P. aeruginosa* is a common Gram-negative, citrate, catalase, and oxidase positive bacterium. It is found in soil, water, skin flora, and most man-made environments throughout the world. It flourishes not only in normal atmospheres, but also in hypoxic atmospheres, and thus known to colonize many natural and artificial environments. It has been reported to cause infections such as blood transfusion-related septicemia (Khabbaz *et al*. 1984 and Scott *et al*. 1988), catheter-related bacteremia (Hsueh *et al*. 1998) and peritonitis in peritoneal dialysis patients (Taber *et al*.1991). Because it thrives on moist surfaces, this bacterium is also found on and in medical equipment, including catheters, causing cross-infections in hospitals and clinics (Balcht *et al*. 1994).

REVIEW OF LITERATURE

We will presumably find ourselves engrossed in the field of nano-biotechnology very soon. It has now become a powerful basic and applied science tool drawing attention of the scientific community towards itself. Nano-biotechnology is defined as a field that uses biological materials to create new devices by applying biological principles to them to form the nano-scale integrated system to transform bio systems. The conventional methods for the synthesis of nanoparticles using chemical means disturb the eco-system posing a serious threat to environment and thus novel methods that promotes the ecological safety are to be explored that may include the use of several living systems for nanoparticles synthesis. A step has been taken to synthesize nano-sized particles of interest with a wide range of biological systems from bacteria to plants and mammals. Most important among them is synthesis using plant extracts which not only is an easy going process but also eliminates the laborious procedure involving the maintenance of microbial cultures. These biological molecules act as reducing agents and oxidizing agents that are significantly safe over chemicals use thus precluding the need of chemical reagents for synthesis. Taking into account the above aspects, biological resources are the best alternative source rather than all the other means for synthesis of nanoparticles (Riddin *et al.* 2006 and Narayana *et al.* 2010). In recent years, biological resources have replaced chemical methods for the synthesis of silver nanoparticles (Bansal *et al*. 2004, 2005; Kumar *et al*. 2007).

Numerous plant resources have been extensively used in the synthesis of silver nanoparticles. Some reports are available on biosynthesis of different metallic nanoparticles and antimicrobial activity using plant materials. Following workers are reported to synthesize different metal based nanoparticles using different plant material extract on the basis of literature survey-

Plant material used	Biosynthesis of nanoparticles	Size of nanoparticles (in nm)	Microbes used for antibacterial assay	Title of the paper	References
Abrus precatorious seed extract (1mM)	ZnO	90 & 500	-	Green synthesis of ZnO Nanoparticles using *Abrus precatorius* seeds extract and their characterization	Vishwakarma, 2014
Benincasa hispida seed extract ($2.5x10^{-4}$ M)	$HAuCl_4$	5-40	*Fusarium oxysporium, Trichoderma viridae*	Biosynthesis of elixir of life (gold nanoparticles) from plants	Singh *et.al*. 2013
Brassica niger seed extract (1mM)	$AgNO_3$	6-20	*Pseudomonas putida, Escherichia coli, Staphylococcus aureus, Bacillus subtilis*	Biofabrication of antibacterial silver nanoparticles from black mustard seed powder and their characterization	Manisha *et. al.* 2014
Carica papaya fruit extract (1mM)	$AgNO_3$	-	*Escherichia coli & Pseudomonas aeruginosa*	Papaya fruit extract: A Potent source for synthesis of bionanoparticles	Maqdoom *et. al.* 2013
Carica papaya fruit extract (1mM)	$AgNO_3$	-	*Escherichia coli, Aspergillus niger*	Bioreduction of silver nanoparticles using different plant extracts and its bioactivity against *E. coli* and *Aspergillus niger*	Choudhary *et. al.* 2014
Carica papaya fruit extract (1mM)	$AgNO_3$	15	*Escherichia coli, Pseudomonas aeuroginosa*	Synthesis of plant-mediated silver nanoparticles using papaya fruit extract and evaluation of their antimicrobial activities	Jain *et. al.*2009
Carica papaya leaf extract (1mM)	$AgNO_3$	25-35	-	Investigation of biogenic silver nanoparticles green synthesized from *Carica papaya*	Konjari *et. al.* 2015

Carica papaya leaf extract (1mM)	$AgNO_3$	5 & 40	*Staphylococcus aureus, Escherichia coli, Bacillus subtilis, Mycobacterium luteus, pseudomonas putida, Klebsiella pneumoniae*	Green synthesis and characterization of *Carica papaya* leaf extract coated silver nanoparticles through X-ray diffraction, electron microscopy and evaluation of bacterial properties and evaluation of bacterial properties	Banala *et. al.* 2015
Coffea Arabica, Trachyspermum ammi & Cuminum cyminum seed extract (0.001 M)	$FeCl_3$	50-100 nm	-	Ecofriendly green synthesis of iron nanoparticles from various plants and spices extract	Pattanayak & Nayak, 2012
Cucurbita lagenaria fruit extract (1mM)	$AgNO_3$	11.2 - 50.12	*Klebsiella pneumoniae* NCIM 2719 & *Clostridium acetobutilicum*	Rapid biosynthesis of silver nanoparticles using bottle gourd fruit extract and potential application as bactericide	Abasaheb *et al*. 2013
Cucurbita pepo leaf extract (1.1 × 10–3M)	$HAuCl_4$	-	-	*Cucurbita pepo* L. extracts as a versatile hydrotropic source for the synthesis of gold nanoparticles with different shapes	Gonnelli *et. al.*2015
Cucurbita pepo leaf extract (1 mM)	HAuCl4	-	*Bacillus cereus, Staphylococcus aureus, Listeria monocytogenes, Escherichia coli, Staphylococcus typhi and Staphyloccus*	Effect of size and shape controlled biogenic synthesis of gold nanoparticles and their mode of interactions against food borne	Chandran *et al.* 2014

			enterica	bacterial pathogens	
Elettaria cardamomum Seed extract	HAuCl4	432.3	-	Green Synthesis of Gold Nanoparticles Using *Elettaria cardamomum* (Elaichi) Aqueous Extract	Pattanayak & Nayak, 2013
Foeniculum vulgare seed extract (8 mM)	$AgNO_3$	11-25	*Pseudomonas aeruginosa, Proteus mirabilis, Escherichia coli, Shigella flexnari* and *Klebsiella pneumoniae*	Rapid green synthesis of silver nanoparticles using seed extract of *Foeniculum vulgare* and screening of its antibacterial activity	Showmya *et al*. 2012
Glycine max seed extract (1mM)	$AgNO_3$	25-50	-	Soyabean seeds extract based green synthesis of silver nanoparticles	Prasad *et. al.* 2014
Jatropha curcas seed extract (1mM)	$AgNO_3$	10.48 ± 2.74	*Escherichia coli, Salmonella paratyphii, bacillus subtilis, Staphyloccus aureus*	Green synthesis and characterization of silver nanoparticles using *Jatropha* seed cake extract	Bose *et. al.* 2012
Lens culinaris seed extract (3mM)	$AgNO_3$	7-18	-	Green synthesis of Ag nanoparticles in the present of *Lens culinaris* seed exudates	Shams *et. al*. 2013
Momordica charantia peel extract (1mM)	$HAuCl_4$	30-100	-	Green synthesis of highly stable gold nanoparticles using *Momordica charantia* as Nano fabricator	Pandey *et. al.* 2012
Momordica charantia leaf extract (1mM)	$AgNO_3$	10-50 nm	*Klebsiella pneumonia*	Biosynthesis of silver nanoparticles by *Momordica*	Samuel *et al*. 2014

				charantia leaf extract: Characterization and their antimicrobial activities	
Momordica charantia fruit extract (1mM)	$AgNO_3$	-	-	Green Synthesis of Silver Nanoparticles Using *Momordica charantia* Fruit Extracts	Nahar *et al*. 2015
Olea europaea seed extract (1mM)	$AgNO_3$	30 ± 6	*Aspergillus niger* *Aspergillus flavus* *Rhizoctonia bataticola*	Green synthesis of silver nanoparticles with high fungicidal activity from olive seed extract	Khadri *et. al* 2013
Pisum satuvum seed extract (3mM)	$AgNO_3$	3-36	*Escherichia coli* and *Candida albicans*	Biosynthesis of silver nanoparticles using plant seeds and their antimicrobial activity	Paul and Yadav, 2015
Sesamum indicum seed extract (2mM)	$AgNO_3$	13	*Escherichia coli*	The Antibacterial Activity of Silver Nanoparticles Produced in the Plant *Sesamum indicum* Seed Extract: A Green Method against Multi-Drug Resistant *Escherichia coli*	Bokaeian *et al*. 2014
Sinapis arvensis Seed extract (0.1 M)	$AgNO_3$	1-35	*Neofusicoccum parvum*	Synthesis of silver nanoparticles using seed exudates of Sinapis arvensis as a novel bioresource, and evaluation of their antifungal activity	Khatami *et al*. 2015

Strychnos potatorum seed and bark extract (0.1 M)	$AgNO_3$	-	*Escherichia coli, Pseudomonas aeruginosa, Proteus vulgaris, Klebsiella pneumoniae, Staphylococcus aureus, Staphylococcus epidermis, Bacillus sp.*	Antibacterial activity and green synthesis of silver nanoparticles using *Strychnos potatorum* seed and bark extract	Packialakshmi *et. al.* 2014
Syzygium cumini seed extract (1mM)	$AgNO_3$	-	-	Biosynthesis of silver nanoparticles from *Sygyium cumini* (L) seed extract and evaluation of their in vitro antioxidant activities	Banerjee *et. al*. 2011
Vitis Vinifera Leaves (1.3x 10-4 M) and Seeds extract ((1.25x 10-4 M)	$HAuCl_4$	13.1 & 8.9	-	Biosynthesis of Gold Nanoparticles Using Extract of Grape (*Vitis Vinifera*) Leaves and Seeds	Ismail *et. al*. 2014

Since no reports are available on the work on seeds of *Carica papaya*, *Momoridica Charantia* and *Cucurbita pepo* where only antibacterial efficacy is observed as well as their other parts like leaf, fruit, stem etc. have been used for nanoparticles synthesis, hence our choice of plant materials will be focused on seeds powder of *Carica papaya, Cucurbita pepo* and *Momoridica Charantia* for screening purpose and selection of plant material giving the best result among all the chosen plant extracts.

OBJECTIVES

1. Purification of pathogenic microbes (*Bacillus subtilis, Bacillus fusiformis, Enterobacter aerogenes, Pseudomonas aeruginosa, Proteus vulgaris and Pseudomonas flourescens*).

2. To collect different plant seeds and their powder preparation for biosynthesis of silver nanoparticles.

3. To screen the production of silver nanoparticles from seeds extract.

4. Characterisation of silver nanoparticles through UV-Vis spectroscopy, XRD, FTIR, EDX and SEM.

5. To test the antimicrobial activity of prepared silver nanoparticles of selected plant material against selected pathogens.

6. To observe the effect of biosynthesized silver nanoparticles with combination of antibiotics against human pathogenic microbes.

METHODOLOGY

1. **Isolation and purification of microbes -** The human pathogenic microbes (*Bacillus subtilis*, *Bacillus fusiformis*, *Enterobacter aerogenes*, *Pseudomonas aeruginosa*, *Proteus vulgaris and Pseudomonas flourescens*) will be obtained from MTCC. The petridishes will be incubated at 37°C for 24 hours. When microbial colonies appeared, it would be transferred to other dishes for the purification. Sub culturing of respective colonies till pure culture is obtained would carry out by the purification. NAM (Nutrient agar medium) will be used. Chemical constituent of NAM medium: 5 g peptone, 3 g beef extract, 5 g sodium chloride, 20 g agar in a litre solution.
2. **Collection of plant material and Preparation of plant seeds extract -** Seeds will be collected from different fruits and vegetables shops from market, Agra and then dried for about one month in shed. Dried seeds of *Carica papaya*, *Cucurbita pepo* and *Momordica charantia* will be grinded into fine powder. Required amount of powder will be dissolved in distill water and will be placed under water bath at 100º C for about 30 minutes for boiling the mixture. After boiling, the sample will be allowed to filter through whatman's filter paper of pore size 5-8 µm to obtain the extract and after filteration centrifugation will be done to remove further biomass and then will be stored in freeze (Banerjee *et al*. 2011 and Khadri *et al*.2013).
3. **Synthesis and screening of silver nanoparticles -**1 mM, 2mM and 3 mM concentrations of silver nitrate ($AgNO_3$) respectively would be prepared in distill water and will be used for synthesis of silver nanoparticles. Seeds extract will be added to each concentration of aqueous solution of silver nitrate solution for reduction of Ag^+ ions into Ag and will be observed for production of AgNPs at different time intervals over a period of 24 hours (Bankara *et al*. 2010, Banerjee *et al*. 2011 and Khadri *et al*. 2013).

   The biochemical reaction of plant extract with $AgNO_3$ leads to the formation of AgNPs by following reaction showing mechanism of biosynthesis of silver nanoparticles:

$$Ag^+NO_3^- + \text{Plant extract} \rightarrow \quad Ag^0NPs + \text{byproducts}$$

4. **Characterisation of silver nanoparticles by UV-Visible spectroscopy, XRD, FTIR, EDX and SEM–** The reduction of pure Ag^+ ions will be monitored by measuring the UV-Vis spectrum of the reaction mixture after diluting a small aliquot of sample into distilled water (Shankar *et al*. 2003). Purified silver particles by repeated centrifugation will be collected for the analysis of their sturcture and composition by XRD. FTIR analysis will be done to confirm that bioreduction of Ag^+ ions to silver nanoparticles is due to the reduction by capping material of plant extract (Bansal *et al*. 2005 , Huang *et al*. 2007 and Sanghi *et al*. 2009) . EDX will confirm the presence of elemental silver and shape of the nanoparticle. For detection of the size of the nanoparticle SEM will be used (Bar *et al*. 2009 and Sharma *et al*. 2009).
5. **Antibacterial assays-** The selected plant material on the basis of above results will be subjected to antibacterial assay against selected pathogens including *Bacillus subtilis*, *Bacillus fusiformis*, *Enterobactor aerogenes, Proteus vulgaris, Pseudomonas flourescens and Pseudomonas aueruginosa* with certain antibiotics, Pure plant seeds extract and Pure $AgNO_3$ (1mM, 2mM and 3 mM) as control. For measuring the minimum inhibitory concentration, Disc diffusion method and Agar well diffusion method will be performed (Gonnellia *et al*. 2006, Maqdoom *et al*. 2013 and Hidalgo *et al*. 1998). So here the selected plant material *Carica papaya* subjecting upon antibacterial assay against the above selected pathogens exhibit good bacteriostatic and bactericidal activity.
6. **Antibacterial assay for combination of antibiotics with biosynthesized silver nanoparticle -** Low dose of antibiotics will be added with the biosynthesized silver nanoparticles solution to observe the effect on the growth of pathogenic bacteria so as to compare the antibacterial efficacy of silver nanoparticles with antibiotics and without antibiotics (Nanda *et al.* 2014).

SIGNIFICANCE

Plant derivatives derived metal nanoparticles are the hottest topic being studied in the last two decades. This production of metallic nanoparticles is induced by the metabolites present in plants. Thus offering the large scale production of nanoparticles in an eco-friendly manner from plant crude extracts and purified metabolites. There are numerous fields which include the use of plant mediated nanoparticles such as therapeutics, renewable energy, pharmaceuticals, and other commercial products. Various diseases can be diagnosed and treated with these plant mediated metallic nanoparticles with great potency and also prevents side effects up to a large extent.

Here, synthesis of metallic nanoparticles with the use of plant seeds along with their microbial property and their potential applications in treatment of various kinds of diseases are highlighted. Idiosyncratic physical and chemical properties of metal nanoparticles make them extremely valuable for environmental biotechnological applications. Overall, much of the efforts are still required for the improvement in the field of nanotechnology for more advanced and vast applications and continuous research is needed. If the exact mechanisms for the formation of nanoparticles are known in a better way, it will be easier to manipulate it to control the shape, size and disparity of the nanoparticles.

BIBOLIOGRAPHY

Abasaheb Ramchandra, Nalwade Swati, Shinde Sudhir, Gajanan Laxman Bhor, Namdeo Bhagwan Admuthe, Shinde Dagadu Sambhaji and Gawade Vasant Vaishali, 2013. Rapid biosynthesis of silver nanoparticles using bottle gourd fruit extract and potential application as bactericide. *Research in Pharma.* 3(3): 22-28.

Ahmad, Mukherjee P, Mandal D, Senapati S, Khan MI, Kumar R, Sastry M, 2002. Enzyme Mediated Extracellular Synthesis of CdS Nanoparticles by the Fungus, *Fusarium oxysporum. Am. J. Chem. Soc.* 124: 12108–12109.

Ahmad, Senapati S, Khan MI, Kumar R, Sastry M**,** 2003. Extracellular Biosynthesis of Monodisperse Gold Nanoparticles by a Novel Extremophilic Actinomycete, *Thermomonospora* sp. *Langmuir.* 19: 3550–3553.

Balcht, Aldona, Smith, Raymond, 1994. *Pseudomonas aeruginosa:* Infections and Treatment. *Informa Health Care*. 83–84.

Banala Rajkiran Reddy, Veera Babu Nagati, Pratap Reddy Karnati, 2015. Green synthesis and characterization of *Carica papaya* leaf extract coated silver nanoparticles through X-ray diffraction, electron microscopy and evaluation of bacterial properties and evaluation of bacterial properties. *Saudi J. Biolo. Sci.* 22(55): 637-644.

Banerjee Joyita & Narendhirakannan RT, 2011. Biosynthesis of silver nanoparticles from *Syzygium cumini (l)* seed extract and evaluation of their in vitro antioxidant activities. *Digest J. Nanomat. Biostruc.* 6(3): 961.

Bankara A, Joshi B, Ravi Kumara A, Zinjardea S, 2010. Banana peel extract mediated novel route for the synthesis of silver nanoparticles**.** *J Colloids Surf. A: Physicochem. Eng.* 368: 58–63.

Bansal V, Rautaray D, Ahmad A, Sastry M, 2004. Biosynthesis of zirconia nanoparticles using the fungus *Fusarium oxysporum. J. Materials Chem*. 14: 3303-3305.

Bansal V, Rautaray D, Bharde A, Ahire K, A Sanyal, A Ahmad, 2005. Fungus-Mediated Biosynthesis of Silica and Titanium Particles. *J. Materials Chem.* 26(15): 2583-2589.

Bar H, Bhui DK, Sahoo GP, Sarkar P, Pyne S, Misra A, 2009. Green synthesis of silver nanoparticles using seed extract of *Jatropha curcas*. *J Colloids Surf. A: Physicochem. Eng.* 34 (8): 212–216.

Bharde AA, Parikh RY, Baidakova M, Jouen S, Hannoyer B, Enoki T, Prasad BLV, Shouche YS, Ogale S, Sastry M, 2008. Bacteria-Mediated Precursor-Dependent Biosynthesis of Super paramagnetic Iron Oxide and Iron Sulfide Nanoparticles. *Langmuir*. 24: 5787–5794.

Bokaeian Mohammad, Sheikh Mousa, Hassanshahian Mehdi, Saeide Saeidi, Sahraei Shahla, 2014. The Antibacterial Activity of Silver Nanoparticles Produced in the Plant *Sesamum indicum* Seed Extract: A Green Method against Multi-Drug Resistant *Escherichia coli*. *Int. J. Enteric Pathog.* 2(2).

Bose Anjali, Keharia Haresh and Deshpande MP, 2012. Eco-friendly phyto-synthesis of silver nanoparticles using *Jatropha* seedcake extract. *Industrial Crops and Products.* 30(12).

Chandran Krishnaraj, Seonhwa Song, Soon-II Yun, 2014. Effect of size and shape controlled biogenic synthesis of gold nanoparticles and their mode of interactions against food borne bacterial pathogens. *Arabian J. Chem. 561-574*.

Choudhary RS, Bhamare NB, Mahure BV, 2014. Bioreduction of Silver Nano-particles using different plant extracts and its bioactivity against *E. coli* and *A. niger*. *Agri. J. Veterinary sci.* 7(7): 07-11.

Drexler & Smalley, 2003. Nanotechnology: make the case for and against molecular assemblers. *Chemical & Engineering News (Amer. Chem. Society)*. 81 (48): 37-42.

Ferdinand Cohn, 1872. Untersuchungen uber Bacterian. *Beitrage zur Biologie der Pflanzen*. 127-224.

Frank JF, Doyle MP, Beuchat LR, Montville TJ, 1997. Milk and dairy products. *In Food Microbiology, Fundamentals and Frontiers ed. ASM Press Washington*: 101.

Gibb AP, Martin KM, Davidson GA, Walker B, Murphy WG, 1995. Rate of growth of *Pseudomonas fluorescens* in donated blood. *Clin. J. Patho.* 48(8): 717–8.

Gonnellia Cristina, Cacioppoa Federica, Giordanobc Cristiana, Capozzolib Laura, Salvaticibc Cristiana, Salvaticib Maria Cristina, Colzia Ilaria, Bubbad Del Massimo, Ancillottid Claudia & Brett DW, 2006.

A Discussion of Silver as an Antimicrobial Agent: Alleviating the Confusion. *Ostomy Wound Manage.* 1(52): 34-41.

Grossmann ME, Mizuno NK, Dammen ML, Schuster T, Ray A, Cleary MP, 2009. Eleostearic Acid inhibits breast cancer proliferation by means of an oxidation-dependent mechanism. *Cancer Prev. Res. (Phila).* 2(10):879-886.

Hidalgo E and Domínguez C, 1998. Study of Cytotoxicity Mechanisms of Silver Nitrate in Human Dermal Fibro- blast. *Toxicology Letters.* 3(98): 169- 179.

Hsueh PR, 1998. Outbreak of *Pseudomonas fluorescens* bacteremia among oncology patients. *Clin. J. Microbio.* 36: 2914–2917.

Huang J, Li Q, Sun D, Lu Y, Su Y, Yang X, 2007. Biosynthesis of Silver and Gold Nanoparticles by Novel Sundried *Cinnamomum Camphora* Leaf. *Nanotechn*. 10(18): 105-104.

Ismail Eman H, Khalil Mostafa MH, Seif Fozia A Al, Fatma E-Magdoub, 2014. Biosynthesis of Gold Nanoparticles Using Extract of Grape (*Vitis Vinifera*) Leaves and Seeds. *Progress in Nanotech. Nanomate.* 1(3): 1-12.

Jain Devendra, Kumar Daima Hemant, Kachhwaha Sumita & Kotharia SL, 2009. Synthesis of plant-mediated silver nano-particles using papaya fruit extract and evaluation of their anti-microbial activities. *Digest J. Nanomate. Biostruc*. 4(4): 723 – 727.

Jay, JM, 2000. Taxonomy, role, and significance of microorganisms in food. *In Modern Food Microbiology, Aspen Publishers, Gaithersburg MD*: 13.

Jha K, Prasad K, Prasad K, 2009. A green low-cost biosynthesis of Sb_2O_3 nanoparticles. *Biochem. J. Eng.* 43: 303–306.

Karunakar Kudle Rao, Manisha R Donda, Alwala Jahnavi, Koyyati Rama, Nagati Veerababu, Merugu Ramchander, Prashanthi Y, Pratap Rudra MP, 2012. Biofabrication of silver nanoparticles using *Cuminum cyminum* through microwave irradiation. *Inter. J. Nanomat. Biostruc*. 2(4): 65-69.

Khabbaz RF, 1984. *Pseudomonas fluorescens* bacteremia from blood transfusion. *Am. J. Med*. 76: 62–68.

Khadri Habeeb, Alzohairy Mohammad, Janardhan Avilala, Arthala Kumar Praveen, Narsimha Golla, 2013. Green Synthesis of Silver Nano-particles with High Fungicidal Activity from Olive Seed Extract. *Advan. in Nanopar*. 2: 241-246.

Khatami Mehrdad, Shahram Pourseyedi, Khatami Mansour, Hadi Hamidi, Mehrnaz Zaeifi and Lida Soltani, 2015. Synthesis of silver nanoparticles using seed exudates of *Sinapis arvensis* as a novel bioresource and evaluation of their antifungal activity. *Bioresou. and Bioproc.* 2:19.

Konjari S Reshma, Jacob A Anju, Jayanti S, Ramalingam C, Ethiraj Sagadevan Anita, 2015. Investigation of biogenic silver nanoparticles green synthesized from *Carica papaya*. *Inter. J. Pharma. Sci.* 7(3): 107-110.

Koyyati Rama, Nagati Veerbabu, Merugu Ramchander, Mnathurpadigya, 2014. Antibacterial activity of silver nanoparticles using *Amaranthus viridis* twig extract. *Inter. J. Res. Pharm. Sci.* 5(1): 32-39.

Koyyati Rama, Nagati Veerbabu, Merugu Ramchander, Mnathurpadigya, 2013. Biological synthesis of silver nanoparticles using *Raphanus sativus* var. *longipinnatus* leaf extract and evaluation of their antioxidant and antibacterial activity. *Inter. J. Med. Pharma. Sci*. 3(4): 89-100.

Kumar SA, Abyaneh MK, Gosavi SW, Kulkarni SK, Pasricha R, Ahmad A, Khan MI, 2007. Nitrate reductase-mediated synthesis of silver nanoparticles from $AgNO_3$. *Biotechnology Letters* 29: 439-445.

Lee J, Kim G, Lee HG, 2010. Characteristics and Antioxidant Activity of *Elsholtzia splendens* Extract-Loaded Nanoparticles. *Agric. J. Food Chem*. 58: 3316–332.

Li X, XH, Chen Z, Chen G, 2011. Biosynthesis of nanoparticles by microorganisms and their applications. *J Nanomater*: 1-16.

Li, SS, Xie Y, A, Yu, Qui X, Zhang L, L Zhang, 2007. Green synthesis of silver nanoparticles using *Capsicum annum* L. extract. *Green Chemi*. 9: 852-858.

Manisha Donda R, Kudle Rao Karunakar, Alwala Jahnavi, Miryala Anila, Sreedhar B and Pratap Rudra MP, 2013. Synthesis of silver nanoparticles using extracts of *Securinega Leucopyrus* and evaluation of its antibacterial activity. *Int. J. curr. Sci*. 7: 1-8.

Manisha DR, Merugu Ramchander, Pratap Rudra MP, 2014. Bio-fabrication of antibacterial silver nanoparticles from black mustard seed powder and their characterization. *Inter. J. Nanomat. Biostruc*. 4(3): 58-62.

Maqdoom Farooqui, Sabeen Hashmi & Shaikh Zarina, 2013. Papaya fruit extract: a potent source for synthesis of bio-nanoparticle. *J. Envt. Research and Development.* 7 (4).

Morton JF, Miami, FL, 1987. Papaya: New CROP, the New Crop Resource Online Program, Center for New Crops & Plant Products, Purdue University. *In: Fruits of warm climates,* 336–346.

Nadagouda MN, Speth TF, Varma RS, 2011. Microwave-assisted green synthesis of silver nanostructures. *Accounts Chem. Res.* 44: 469-478.

Nahar MK, Zakaria Z, Hashim U, MF Bari, 2015. Green Synthesis of Silver Nanoparticles Using *Momordica charantia* Fruit Extracts. *Advan. Mater. Res*.1109: 35-39.

Nalvothula Raju, Nagati Babu Veera, Koyyati Rama, Merugu Ramchander, Rudra Padigya Manthur Pratap, 2014. Biogenic Synthesis of Silver Nanoparticles Using *Tectona grandis* Leaf Extract and evaluation of their Antibacterial Potential. *Inter. J. of Chem. Tech. Research*. 6(1): 293-298.

Nanda Anima, Amin M Bhat, Nayak BK, 2015. Enhanced antibacterial activity of commercial antibiotics using AgNPs synthesized from *Aspergillus niger*. *Der. Pharmacia. Lettre.* 7(5): 281-285.

Narayana KB and N Sakthivel, 2010. Biological Synthesis of Metal Nanoparticles by Microbes. *Advan. Colloids Interface Sci*. 1-2(156): 1-13.

Packialakshmi N, Suganya C, Guru V, 2014. Antibacterial activity and green synthesis of silver nanoparticles using *strychnos potatorum* seed and bark extract. *Asian J. Phytomed. Clin. Research.* 2(3) 127 – 138.

Palleroni, NJ, Krieg, NR, Holt JG, 1984. Pseudomonadaceae. Bergey's Manual of Systematic Bacteriology (editors) Baltimore: *The Williams and Wilkins Co*. 141 – 199.

Pandey Sunil, Goldie Oza, Ashmi Mewada and Madhuri Sharon, 2012. Green Synthesis of Highly Stable Gold Nanoparticles using *Momordica charantia* as nano fabricator. *Arch. Appl. Sci. Res.* 4 (2):1135-1141.

Pattanayak Monalisa and Nayak PL, 2013. Green Synthesis of Gold Nanoparticles Using *Elettaria cardamomum* (Elaichi) Aqueous Extract. *World J. Nano Sci. Tech*. 2(1): 01-05.

Pattanayak Monalisa and Nayak PL, 2012. Ecofriendly green synthesis of iron nanoparticles from various plants and spices extract. *Inter. J. Plant Animal Envt. Sci.* 3(1):68-78.

Paul S Nilesh and Yadav P Raman, 2015. Biosynthesis of silver nanoparticles using plant seeds and their antimicrobial activity. *Biomed. J. Pharma. Sci.* 5(45): 26-28.

Plante IJL, Zeid TW, Yangab P, Mokari T, 2010. Synthesis of metal sulfide nanomaterials via thermal decomposition of single-source precursors. *Mater. J. Chem.* 20: 6612-6617.

Prasad Ch. & Venkateshwarlu Ponneri, 2014. Soyabean seeds extract based green synthesis of silver nanoparticles. *Indian J. advances chem. sci*. 2(3): 208-211.

Raveendran, PF, J & Wallen, SL, 2003. Completely. Green Synthesis and Stabilization of metal nanoparticles. *Amer. J. Chem. Society*. 125(46): 13940-13941.

Ray B, 1996. Spoilage of Specific food groups. I*n Fundamental Food Microbiology, CRC Press, Boca Raton FL:* 220.

Riddin TL, Gericke M and Whiteley CG, 2006. Analysis of the Inter and Extracellular Formation of Platinum Nanoparticles by *Fusarium oxysporum* sp. *Lycopersicum* using Surface Response Methodology. *Nanotechnology*. 14(17): 3482-3489.

Samuel Alwin David , Kanagasabai Muruganandam Ponvel, Mohamed Anis Fathima, Solaiappan Anita, Jeyapaul Ashli and Alaguvel Athilakshmi, 2014. Biosynthesis of silver nanoparticles by *Momordica charantia* leaf extract: Characterization and their antimicrobial activities. *Nat. J. Prod. Plant Resour*. 4(6):1-8.

Sanders WE, Sanders CC, 1997. *Enterobacter* sp.: pathogens poised to flourish at the turn of the century. *Clin. Microbiol. Rev*. 10(2): 220–41.

Sanghi R and Verma P, 2009. Biomimetic Synthesis and Characterization of Protein Capped Silver Nanoparticles. *Bioresource Techn*. 1(100): 501- 504.

Scott J, 1988. A fatal transfusion reaction associated with blood contaminated with *Pseudomonas fluorescens*. *Vox. Sang*. 54: 201–204.

Shahverdi AR, Minaeian S, Shahverdi HR, Jamalifar H, Nohi A, 2007. Rapid synthesis of silver nanoparticles using culture supernatants of *Enterobacteria:* A novel biological approach. *Process Biochem*. 42: 919–923.

Shams Somaye, Pourseyedi Shahram, Raisi Moslem, 2013. Green synthesis of Ag nanoparticles in the present of *Lens culinaris* seed exudates. *Intl. J Agri. Crop Sci.* 5(23): 2812-2815.

Shankar S, Ahmad A and Sastry M, 2003. *Geranium* Leaf Assisted Biosynthesis of Silver Nanoparticles. *Biotech. Progress.* 6(19): 1627-1631.

Showmya Jerlin J, Harini K, Pradeepa M, Thiyagarajan M, Manikandan R, Venkatachalam P, Geetha N, 2012. Rapid green synthesis of silver nanoparticles using seed extract of *Foeniculum vulgare* and screening of its antibacterial activity. *Society for Biol. Biotech.* 13(1-2):31-38.

Singh Kumar Pankaj and Subir Kundu, 2013. Biosynthesis of elixir of life (gold nanoparticles) from plants. *J. Environ. Nanotechn*. 1(2): 52-62.

Taber TE, Hegeman TF, York SM, Kinney RA, Webb DH, 1991. Treatment of *Pseudomonas* infections in peritoneal dialysis patients. *Perit. Dial. Int*. 11: 213–216.

Torresdey Gardea JL, Gomez E, Peralta-Videa JR, Parsons JG, Troiani H, Jose M, 2003. *Alfalfa* Sprouts: A Natural Source for the Synthesis of Silver Nanoparticles. *Langmuir.* 19: 1357-1361.

Vishwakarma Kavita, 2014. Green synthesis of ZnO nanoparticles using *Abrus precatorius* seeds extract and their characterization. *Digest J. nanomat. Biostruc.*4 (1): 159-166.